Role of Inducible Nitric Oxide Synthase in the Pulmonary Vascular Response to Birth-Related Stimuli in the Ovine Fetus

Robyn L. Rairigh, Thomas A. Parker, D. Dunbar Ivy, John P. Kinsella, I-Da Fan, Steven H. Abman

Abstract—To determine whether type II nitric oxide synthase (NOS II) contributes to the NO-mediated fall in pulmonary vascular resistance (PVR) at birth, we studied the effects of selective NOS II antagonists N-(3-aminomethyl)benzylacetamidine dihydrochloride (1400W) and aminoguanidine (AG) and a nonselective NOS antagonist, nitro-L-arginine (L-NA), during mechanical ventilation with low FiO₂ (<10%), high FiO₂ (100%), and inhaled NO (20 ppm) in 23 near-term fetal lambs. Intrapulmonary infusions of AG, 1400W, and L-NA increased basal PVR before delivery (P<0.05). In control animals, ventilation with low and high FiO₂ decreased PVR by 62% and 85%, respectively. Treatment with AG and 1400W attenuated the fall in PVR by 50% during ventilation with low and high FiO₂ (control versus treatment, P<0.05 for each intervention). L-NA treatment attenuated the fall in PVR during ventilation with low and high FiO₂ to a similar degree as the NOS II antagonists. To test the selectivity of the NOS II antagonists, we studied the effects of acetylcholine and inhaled NO in each study group. Acetylcholine-induced pulmonary vasodilation remained intact after treatment with selective NOS II antagonists but not after treatment with nonselective NOS blockade with L-NA. In contrast, the response to inhaled NO was similar between treatment groups. We conclude that selective NOS II inhibition is as effective as nonselective NOS blockade in attenuating pulmonary vasodilation at birth and speculate that NOS II activity contributes to NO-mediated pulmonary vasodilation at birth. We additionally speculate that stimulation of the airway epithelium by rhythmic distension and increased FiO₂ may activate NOS II release at birth. (Circ Res. 2001;88:721-726.)

Key Words: pulmonary circulation ■ pulmonary hypertension ■ nitric oxide ■ nitric oxide synthase ■ persistent pulmonary hypertension of the newborn

Nitric oxide (NO) is produced by NO synthase (NOS) during the conversion of L-arginine to L-citrulline and plays an important role in the regulation of vascular tone in the perinatal pulmonary circulation. At birth, the lung circulation undergoes a marked fall in pulmonary vascular resistance (PVR), which is partly attributable to stimulation of NOS activity. In the fetus, inhibition of NOS with L-arginine analogues increases basal PVR, blocks the pulmonary vasodilator response to shear stress, enhances the myogenic response, and attenuates the vasodilator response to several pharmacological and physiological stimuli. In addition, NOS inhibition attenuates the fall in PVR at birth, suggesting that NO contributes to pulmonary vasodilation during the transition to postnatal life.

Three distinct isoforms of NOS have been identified: type I, or neuronal (NOS I); type II, or inducible (NOS II); and type III, or endothelial (NOS III). Although past studies have presumed that the NOS III isoform is the predominant source of NO production in the perinatal pulmonary circulation, the arginine analogues that were used to inhibit NOS activity in these physiological studies were not isoform-selective.

NOS II has been studied extensively in experimental models of circulatory shock, autoimmune disease, hypoxia, and chronic inflammation. In addition to the endothelial NOS isoform, recent studies have shown that the NOS I and NOS II isoforms are also expressed in the fetal lung. Our laboratory has previously demonstrated that the NOS II isoform is constitutively expressed predominately in airway epithelium and vascular smooth muscle in the late-gestation ovine fetal lung and that intrapulmonary infusions of selective NOS II antagonists increase basal PVR in the late-gestation fetus. Recently, it was shown that selective NOS II antagonists attenuate shear stress–induced pulmonary vasodilation caused by acute compression of the ductus arteriosus, whereas nonselective blockade with nitro-L-arginine (L-NA) completely blocked this response. These findings suggest that NOS II produces NO in the normal fetal lung under basal conditions and contributes in part to in utero NO production in response to acute shear stress. Whether NOS II activity plays an important role in the release of NO during the normal transition of the pulmonary circulation at birth is unknown.

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From the Pediatric Heart Lung Center and the Sections of Neonatology (R.L.R., T.A.P., J.P.K.), Cardiology (D.D.I.), and Pulmonary and Critical Care Medicine (S.H.A.), Department of Pediatrics, University of Colorado School of Medicine, Denver, Colo.
Correspondence to Steven H. Abman, MD, Pulmonary Medicine, B395, The Children’s Hospital, 1056 E Nineteenth Ave, Denver, CO 80218-1088.
E-mail steven.abman@UCHSC.edu
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To test whether NOS II activity contributes to the NO-mediated effects of 2 selective NOS II antagonists, aminoguanidine (AG) and N-(3-aminomethyl) benzylacetamide dihydrochloride (1400W), during rhythmic distension of the lung (by mechanical ventilation with low FIO₂ [<10%] to maintain arterial P₀₂ [at fetal values]) and with exposure to high (100%) FIO₂ in the late-gestation fetal lamb. We compared these responses with the effects of L-NA, a nonselective NOS antagonist, during ventilation with low and high FIO₂. Finally, we compared the pulmonary vascular effects of the selective NOS II and nonselective NOS antagonists on the vasodilator responses to acetylcholine (ACh), an endothelium-dependent agonist, and inhaled NO, which causes vasodilation independent of NOS activity.

Materials and Methods

Surgical Preparation and Physiological Measurements

All procedures and protocols were reviewed and approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center. Mixed breed (Columbia-Rambouillet, Nebeker Ranch, Lancaster, Calif) pregnant ewes between 138 and 140 days’ gestation (term=147 days) were fasted 24 hours before surgery. Ewes were sedated with intravenous pentobarbital sodium (2 to 4 mg) and anesthetized with 1% tetracaine hydrochloride (3 mg) by lumbar puncture. Ewes were kept sedated with pentobarbital but breathed spontaneously throughout surgery. Under sterile conditions, the left forelimb of the fetal lamb was delivered through a uterine incision. A skin incision was made under the left forelimb after local infiltration with 1% lidocaine. Polyvinyl catheters were inserted into the axillary artery and vein and advanced into the ascending aorta (Ao) and superior vena cava, respectively. A left axillary to sternal thoracotomy at the 5th to 6th intercostal space exposed the heart and great arteries. Polyvinyl catheters were inserted into the left pulmonary artery (LPA), main pulmonary artery (MPA), and left atrium (LA) by direct puncture and secured in position by purse-string sutures. A 6-mm ultrasonic flow transducer was placed around the LPA to measure blood flow to the left lung. The Ao, MPA, and LA were connected to a computer-driven pressure transducer and recorder. The flow transducer cable was attached to an internally calibrated flowmeter for continuous measurements of LPA flow. The absolute values of flows were determined from phasic blood flow signals, as previously described.²²,²³ PVR in the left lung was calculated by the following equation: PVR (mm Hg/mL per min) = mean MPA pressure—mean LA pressure/LPA blood flow. Arterial blood gas tensions, pH, hemoglobin, and oxygen saturation were measured from blood samples that were drawn from the Ao catheter and measured at 39.5°C with a blood gas analyzer and hemoximeter.

Drug Preparation

All drugs were freshly prepared on the day of study and included AG, 1400W, ACh, and U46619 dissolved in saline before use. L-NA was initially dissolved in 1 mol/L HCL before dilution with normal saline and titration of pH to 7.4 with NaOH. Doses for each drug except 1400W were based on previously published studies.¹,²,³,⁶,¹²,¹³,¹⁴,¹⁶,¹⁷,¹⁹,²¹,²²,²³ The dose of 1400W was determined from preliminary experiments and published studies showing that 1400W is 5 to 10,000 times more selective for type II than type III NOS.²⁶–²⁸

Experimental Design

Studies were performed after at least 60 minutes of recovery from surgery. The identical protocol was followed for each study group: (1) control (sterile saline; n=4 animals; mean gestational age, 139±2 days); (2) LNA treatment (20 mg; n=7 animals; mean gestational age, 139±2 days); (3) AG treatment (120 mg; n=8 animals; mean gestational age, 139±2 days); (4) 1400W treatment (0.12 mg; n=4 animals; mean gestational age, 139±2 days). Hemodynamic measurements were recorded at 10-minute intervals throughout the study period. After 30 minutes of stable baseline hemodynamic measurements, one of the study drugs or saline was infused into the LPA (0.2 mL/min for 10 minutes). Pancuronium bromide (0.5 mg) was infused immediately before tracheostomy and insertion of a 5-mm internal diameter endotracheal tube. Mechanical ventilation with low (5% to 10%) FIO₂ was initiated to achieve lung inflation and ventilation without changing fetal arterial P₀₂. Initial ventilator settings included a rate of 20 breaths per minute, peak inspiratory pressure of 30 cm H₂O, positive end-expiratory pressure of 4 cm H₂O, and an inspiratory time of 1 second. Ventilator rate and peak inspiratory pressure were varied as necessary to maintain PCO₂ at 35 to 40 mm Hg. Animals were ventilated sequentially with low FIO₂ (for 60 minutes), high FIO₂ (for 30 minutes), and high FIO₂ with inhaled NO (20 ppm; 10 minutes). Selection of the time intervals for each intervention was based on past experience, which showed that more time is needed during the initial ventilation period to more gently inflate the lung for optimal lung recruitment without injury and to allow absorption of fetal lung liquid.¹³ Vasodilation to increased FIO₂ is stable after 30 minutes, whereas the response to inhaled NO is more rapid (10 minutes). The response to ACh (1.5 ppm/min for 10 minutes) was studied in each group after ventilation with low FIO₂ To determine the effects of nonspecific pulmonary vasoconstriction to birth-related stimuli, U46619, a thromboxane analogue, was infused at a dose of 0.01 to 0.25 μg/mL per min, which increases basal PVR by 50%.²¹ Data are presented as mean±SEM. Statistical analysis was performed with the Statview 4.5 and Super ANOVA software packages (Abacus Concepts). Comparisons were made by using univariate repeated measures by linear contrast analysis. Post hoc analysis was performed with Newman-Keuls testing. A value of P<0.05 was considered significant.

Results

Vasodilator Response to Birth-Related Stimuli and Inhaled NO in the Late-Gestation Ovine Fetus

In control animals, infusion of normal saline had no effect on baseline hemodynamics or arterial blood gas tension before delivery (Figures 1 through 3 and Tables 1 and 2). Fetal arterial P₀₂ did not change during the 60-minute ventilation period with low FIO₂ (<10%), but LPA blood flow increased from 84±7 to 242±39 mL/min (P<0.001; Figure 1). PVR decreased during ventilation with low FIO₂ (P<0.001; Figure 2), and pulmonary artery pressure (PAP), aortic pressure (AoP), left atrial pressure (LAP), and heart rate did not
change (Figure 3, Table 2). During the 30-minute ventilation period with high FIO₂, LPA blood flow increased 1.5-fold (P < 0.001; Figure 1), and mean PAP and PVR progressively decreased (P < 0.01; Figures 2 and 3). AoP, LAP, and heart rate did not change during the ventilation with high FIO₂ (Table 2). Inhaled NO (20 ppm) did not cause an additional increase in LPA blood flow, decrease in PVR, or decrease in PAP in the control group (Figures 1 through 3). Baseline peak inspiratory pressure was progressively decreased during the study period, from 30 to 26±3 cm H₂O (P < 0.05), but other ventilator settings (rate, positive-end expiratory pressure, and inspiratory time) remained constant. Ventilator settings were not different between study groups at each time point.

Effects of Nonselective NOS Blockade on the Vasodilator Response to Birth-Related Stimuli and Inhaled NO in the Late-Gestation Ovine Fetus

L-NA infusion into the LPA decreased LPA blood flow (P < 0.01; Figure 1) and increased PVR (P < 0.001; Figure 2), mean PAP (P < 0.01; Figure 3), and mean AoP (P < 0.01; Table 2). Ventilation with low FIO₂ increased LPA blood flow from 79±7 to 136±7 mL/min (P < 0.01; Figure 1). Although LPA blood flow increased during ventilation with low FIO₂ of the L-NA treatment group, LPA flow remained less than half of flow achieved in the control group (P < 0.001; Figure 1). PVR progressively decreased during the ventilation period with low FIO₂, but PVR in the L-NA treatment group was 2-fold higher than PVR in the control group (P < 0.001; Figure 2). PAP, AoP, LAP, and heart rate did not change during ventilation with low FIO₂ (Figure 3, Table 2).

**TABLE 1. Effects of Nonselective Blockade and Selective NOS II Blockade on Arterial Blood Gas Tensions, pH, Hemoglobin, and Oxygen Saturation**

<table>
<thead>
<tr>
<th></th>
<th>pH units</th>
<th>P O₂, mm Hg</th>
<th>P CO₂, mm Hg</th>
<th>Hemoglobin, g/dL</th>
<th>Saturation, %</th>
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<td><strong>Control</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>7.29±0.03</td>
<td>20±3</td>
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<td>51±9</td>
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<td>Ventilation (low FIO₂)</td>
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<td>53±6</td>
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<td>Ventilation (high FIO₂)</td>
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<td>100</td>
</tr>
<tr>
<td>NO (20 ppm)</td>
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<td>335±52</td>
<td>34±2</td>
<td>7±1</td>
<td>100</td>
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<td></td>
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<tr>
<td>Baseline</td>
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<td>42±3</td>
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<td>60±3</td>
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<td>Ventilation (high FIO₂)</td>
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<tr>
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<tr>
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<td>NO (20 ppm)</td>
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<td>42±1</td>
<td>7±1</td>
<td>60±7</td>
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<tr>
<td>Ventilation (low FIO₂)</td>
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<td>24±3</td>
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<td>100±6</td>
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<td>332±23</td>
<td>35±3</td>
<td>7±1</td>
<td>100±4</td>
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</table>

Values are mean±SEM.
TABLE 2. Effects of Nonselective Blockade and Selective NOS II Blockade on AoP, LAP, and Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>Mean AoP, mm Hg</th>
<th>Mean LAP, mm Hg</th>
<th>Heart Rate, bpm</th>
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<tr>
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<td>50±2</td>
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<td>175±23</td>
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<tr>
<td>Ventilation (low FIO2)</td>
<td>48±4</td>
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<td>185±30</td>
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<tr>
<td>Ventilation (high FIO2)</td>
<td>53±4</td>
<td>4±3</td>
<td>175±25</td>
</tr>
<tr>
<td>NO (20 ppm)</td>
<td>53±4</td>
<td>3±3</td>
<td>180±20</td>
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<tr>
<td><strong>L-NA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>52±1</td>
<td>4±3</td>
<td>190±32</td>
</tr>
<tr>
<td>Ventilation (low FIO2)</td>
<td>56±1*</td>
<td>3±3</td>
<td>178±30</td>
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<tr>
<td>Ventilation (high FIO2)</td>
<td>57±2*</td>
<td>4±3</td>
<td>184±25</td>
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<tr>
<td>NO (20 ppm)</td>
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<tr>
<td>Baseline</td>
<td>51±1</td>
<td>3±3</td>
<td>170±26</td>
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<td>NO (20 ppm)</td>
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<tr>
<td>NO (20 ppm)</td>
<td>55±1*</td>
<td>3±3</td>
<td>180±25</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05 for control vs treatment.

During ventilation with high FIO2, LPA blood flow increased from 136±7 to 242±11 mL/min but was markedly lower than the control group (P<0.001; Figure 1). Although PVR decreased with ventilation, it remained nearly 2-fold higher than in the control group (P<0.001; Figure 1). PAP progressively decreased during ventilation with high FIO2 (P<0.001; Figure 2) but remained higher than the control group (P<0.05; Figure 3). AoP, LAP, and heart rate did not change during the 30-minute ventilation period with high FIO2 (Table 2). In the L-NA treatment group, ACH-induced pulmonary vasodilation was completely blocked (Figure 4), but the response to inhaled NO remained intact (Figure 5). After 10 minutes of inhaled NO treatment, there was no difference in PVR or PAP between the control group and L-NA treatment group (Figures 2 and 3).

**Figure 4.** Effects of NOS II and nonselective NOS inhibition on ACH-induced pulmonary vasodilation in the late-gestation ovine fetus. Although ACH infusion decreased PVR in the selective NOS II inhibition groups (AG and 1400W), ACH-induced pulmonary vasodilation was completely blocked after treatment with L-NA.

**Figure 5.** Effects of nonselective and selective NOS II blockade on the hemodynamic response to inhaled NO. As shown, brief inhaled NO treatment reduced PVR in the left lung in each study group to values achieved in control lambs.

**Effects of Selective NOS II Blockade on the Vasodilator Response to Birth-Related Stimuli and Inhaled NO in the Late-Gestation Ovine Fetus**

AG infusion before delivery increased PVR (Figure 2), mean PAP (Figure 3), and mean AoP (Table 2) above baseline values and in comparison with the control group. After AG treatment, LPA blood flow increased from 72±4 to 139±14 mL/min (P<0.01; Figure 1) during ventilation with low FIO2 but remained lower than values measured in control animals (P<0.001; Figure 1). However, LPA blood flow during ventilation was not different after selective NOS II (AG) and nonselective NOS (L-NA) blockade (Figure 1). AG treatment decreased PVR during the ventilation period with low FIO2, but PVR remained 2-fold higher than values in the control group (P<0.001; Figure 2). There was no difference in PVR between the selective NOS II (AG) and nonselective NOS (L-NA) blockade (Figure 1). PAP, AoP, LAP, and heart rate did not change during ventilation with low FIO2 (Figure 3, Table 2).

During ventilation with high FIO2, LPA blood flow in the AG treatment group remained lower (P<0.001; Figure 1), and PVR was 2-fold higher than similar measurements in controls (P<0.001; Figure 2). There was no difference in PVR between the selective NOS II blockade (AG) and nonselective NOS blockade (L-NA) groups (Figure 1). In the AG treatment group, inhaled NO increased LPA blood flow (Figure 1), decreased PVR (Figure 2), and decreased PAP (Figure 3). PVR during inhaled NO treatment was similar between each of the study groups. In contrast to the L-NA treatment group, the vasodilator response to ACH was preserved (Figure 4).

Infusion of 1400W before delivery increased PVR, PAP, and AoP above baseline values (P<0.05; Figures 2 and 3). During ventilation with low FIO2, LPA blood flow doubled after 1400W treatment (P<0.05; Figure 1) but remained lower than blood flow in the control group (P<0.001; Figure 1). LPA blood flow was not different between treatment groups (AG, 1400W, and L-NA) (Figure 1). PVR in the 1400W treatment group was 2.3-fold higher than in the control group (P<0.001; Figure 2) but was not different between the other treatment groups (Figure 2). In the 1400W group, LPA blood flow increased during ventilation with high FIO2, but was 2-fold lower than controls (P<0.001; Figure 1). PVR in the 1400W treatment group was 2.9-fold higher than PVR in the control group (P<0.001; Figure 2) and was not different between treatment groups. PAP progressively decreased from 53±2 to 43±2 mm Hg during ven-
tilation with high FiO₂ (P<0.05; Figure 3) but remained significantly elevated above control group (P<0.05). AoP, LAP, and heart rate did not change during the 30-minute ventilation period with high FiO₂ (Table 2). In the 1400W treatment group, inhaled NO (20 ppm) increased LPA blood flow (P<0.05; Figure 1), decreased PVR (P<0.05; Figure 2), and decreased PAP (P<0.05; Figure 3). As observed in the AG group, ACh-induced vasodilation remained intact with 1400W treatment.

To determine whether nonspecific pulmonary vasoconstriction could attenuate the fall in PVR at birth, we also studied the effects of treatment with U46619, a thromboxane analogue, in 3 additional fetal lambs. Continuous infusion of U46619 increased basal PVR by 50% before delivery, matching the change in PVR achieved after treatment with the NOS inhibitors. In contrast to the effects of the NOS inhibitors, the pulmonary vasodilator response to ventilation during low and high FiO₂ after U46619 treatment was identical to measurements in control animals. Infusion of U46619 increased mean PAP (from 51±2 to 62±4 mm Hg), lowered LPA flow (from 128±12 to 80±7 mL/min), and increased PVR by nearly 2-fold (0.40±0.11 to 0.78±0.18 mm Hg/mL per min). During ventilation with low and high FiO₂, PVR decreased to 0.22±0.06 and 0.11±0.04 mm Hg/mL per min, respectively (P<0.05 for each time point versus baseline values; P=NS versus control animals).

Discussion

To determine whether NOS II contributes to the NO-mediated fall in PVR at birth, we studied the hemodynamic effects of 2 selective NOS II antagonists (AG and 1400W) during mechanical ventilation with low (<10%) and high (100%) FiO₂ in the late-gestation fetal lamb. We compared these responses with the effects of L-NA, a nonspecific NOS antagonist. We found that selective NOS II antagonists attenuate the fall in PVR during ventilation with low and high FiO₂ as effectively as nonspecific blockade with L-NA. ACh-induced pulmonary vasodilation remained intact after treatment with AG and 1400W but not after treatment with L-NA, suggesting selectivity of NOS II blockade. In contrast, inhaled NO additionally decreased PVR to levels achieved in control lambs in each of the study groups. These findings suggest that birth-related activation of the NOS II isoform contributes significantly to the release of NO at birth.

NOS II (inducible NOS) has been shown to play a role in pathophysiology of several disorders, such as circulatory shock, autoimmune disease, and chronic inflammation, but few studies have examined its potential physiological roles in normal circulation, especially in the lung. Our study is the first to directly determine the role of NOS II in the regulation of pulmonary vascular tone at birth. Past studies have focused on the NOS III isoform as the likely source of vascular NO production in the perinatal lung, but the arginine analogues that were used to inhibit NOS activity in physiological studies were not isoform-selective. Our data support the hypothesis that the NOS II (inducible) isoform contributes substantially to the normal physiological regulation of the perinatal pulmonary circulation.

Past studies have shown that inhibition of NOS with nonspecific NOS antagonists increases basal PVR, blocks the NO-mediated fall in PVR during shear stress–induced pulmonary vasodilation, and attenuates the fall in PVR at birth. Discrete birth-related stimuli (shear stress, ventilation, and increased oxygen tension) act in part by stimulating the formation of NO by one or more of the 3 known NOS isoforms. Although the endothelial NOS isoform, NOS III, has been presumed to be the major source of NO production at birth, recent studies have demonstrated that the inductive or NOS II isoform produces NO in the normal fetal lung. NOS II mRNA is present in the ovine fetal lung, and NOS II protein is constitutively expressed in airway epithelium and vascular smooth muscle, but positive immunostaining has not been observed in fetal vascular endothelium.

The physiological data presented in this study do not allow the specific identification of the cell type responsible for the NOS II activity that caused vasodilation. However, the striking intensity of immunoreactive NOS II protein in the airway epithelium is interesting. In contrast to the similar effects of selective NOS II and nonspecific NOS blockade in response to ventilation and increased O₂, we have previously reported that NOS II blockade had only a partial effect on shear stress–induced pulmonary vasodilation, whereas L-NA caused complete inhibition of this response. We speculate that rhythmic stretch and increased oxygen exposure of distal airway epithelium may cause activation of NOS II in the airway, leading to vasodilation in response to these birth-related stimuli. Additional work is needed to clarify the cell type that is primarily responsible for NO release during the transition at birth; however, the concept that the effects of changes in alveolar oxygen tension on pulmonary vascular tone may be regulated by arterial NO production of NO has been recently suggested. In this study, alveolar but not vascular hypoxia reduced net lung NO production and increased pulmonary artery pressure in isolated perfused lung preparations.

These findings may also provide an explanation for the observation that NOS III–deficient mice seem to survive the transition at birth. Although NOS III−/− and NOS III+/− mice are more susceptible to the development of pulmonary hypertension to mild hypoxia, basal pulmonary artery pressure is only mildly elevated in these mice. Compensatory upregulation of NOS II may in part account for these findings. Whether the fall in PVR at birth is impaired in NOS III−/− or NOS III+/− mice has not been studied, and such studies are limited by an inability to instrument the fetus for critical physiological measurements.

Past studies have examined the relative selectivity and specificity of NOS II antagonists. AG, a non–amino acid inhibitor, is thought to inhibit NOS II by binding as a ligand to the heme iron at the catalytic site, because AG deactivates other iron- or copper-containing enzymes in this manner. Because electrons from NADPH oxidation eventually reduce heme iron, 1400W binding could alter this flow of electrons and induce inactivation of NOS II. AG and 1400W have been previously shown to have no effect on ACh-induced vasodilation, suggesting that AG and 1400W inhibit NOS II selectively without blocking NOS III. In vitro studies have shown that AG and 1400W are 80- and 5000-fold more potent for NOS II than NOS III, respectively.
In conclusion, we report that selective NOS II blockade attenuates pulmonary vasodilation at birth and that NOS II selective inhibitors were as effective as nonselective NOS inhibitors. These findings support the hypothesis that NOS II is the major NOS isoform responsible for NO-mediated fall in PVR during transition of the pulmonary circulation from fetal to neonatal life. We additionally speculate that exposure of airway epithelium to rhythmic distension with air and increased oxygen tension may stimulate NOS II activity at birth and that diffusion of NO from the distal airway may modulate pulmonary vascular tone.

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References

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Robyn L. Rairigh, Thomas A. Parker, D. Dunbar Ivy, John P. Kinsella, I-Da Fan and Steven H. Abman

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